16 Fungal Contributions to Soil Aggregation and Soil Quality

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Increased public concern for improved environmental quality necessitates determination of the mechanisms important in soil structure formation to decrease runoff and erosion (Karlen et al., 1992). A prerequisite to evaluating soil quality is understanding factors influencing soil structure. Yoder (1937) stated, "Anyone who is conservation minded, and at the same time familiar with the close correlation between poor tilth and high erodibility, is inclined to the view that the possibility of improving soil structure should be investigated to the fullest extent." This statement remains relevant today.

Historically, soil *tilth* has holistically described soil in a conceptual way: ease of tillage, fitness as a seedbed, ability to support plant growth (SCSA, 1982) without recognizing the contributions of the soil biota. Increased interest in the biological contributions to soil tilth has recently resulted in the concept of soil health. A healthy soil is thought to be a biologically active soil with a large and diverse microbial biomass pool. Soil quality encompasses the concepts of soil tilth and soil health; a quality soil would exhibit characteristics of high health and high tilth.

Soil structure has been defined as the size and arrangement of particles and pores in soil (Oades, 1984). Soil structure is created when physical forces (drying, shrink–swell, freeze–thaw, root growth, animal movement, or compaction) mold the soil into aggregates (Paul & Clark, 1988). Soil structure controls the formation and destruction of soil organic matter, soil porosity, and water and solute movement through the soil profile (Elliott & Coleman, 1988).

The formation of soil structure results in aggregate formation. As aggregates become larger, the architecture, arrangement, and stability of the aggregates is important because these factors dictate pore size and continuity. Aggregate breakdown relates closely to infiltration and erodibility in the

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field (Coughlan et al., 1991; Yoder, 1937). If the soil aggregates are unstable during a rainfall event, decreased water infiltration results, due to partial blocking of the pores with aggregate particles (Lynch & Bragg, 1985). As the percentage of water-stable aggregates increase, water infiltration increases with concomitant decrease in erosional losses (Wilson & Browning, 1945). Therefore, aggregate size and stability control the soil pore-size distribution, water infiltration rates, and erosivity.

Numerous research studies indicate the importance of crop residues on promoting aggregation. Crop rotations with low organic matter inputs lead to loss of water-stable aggregates (Boyle et al., 1989; Oades, 1984). Residue management also affects the microbial community composition (Holland & Coleman, 1987; Doran, 1980). However, the actual mechanisms contributing to this loss in aggregation are not well defined.

Numerous writers have implicated soil microorganisms as important factors in soil aggregation and soil structure. A study by Martin and Waksman (1940) indicated that microorganisms are important in promoting soil structure and reducing erosional losses. Hierarchical models of biological contributions to soil structure and aggregation imply that bacteria and fungi promote aggregation in soil. A model developed by Tisdall and Oades (1982) suggests that the bacteria are important at one size-fraction and that fungi are important at another size fraction; however, data to support this model are not included in their paper. A study by Gupta and Germida (1988), which contrasted native and cultivated soil, indicated that the fungal biomass was important in the formation of soil aggregates. In their field study fungal biomass correlated with increases in aggregate mean weight diameter. Their results suggest that the reduced aggregate stability following cultivation can be explained by decreases in total microbial biomass coupled with significant loss of fungal biomass, but no controlled laboratory studies were performed to evaluate this hypothesis.

A deficiency exists in the literature delineating effects of fungi and residue on aggregating soil. The mechanism by which residue stabilizes soil is unclear. While clay–organic matter complexes may increase aggregation at one aggregate-size class, residue additions to soil also provide substrate for increased microbial activity. The purpose of this experiment was to evaluate the potential impact that a soil fungus may have on soil aggregation and to assess the *nonbiological* effects of residue on soil aggregation.

In this laboratory study, the interactions of residue and *Chaetomium* sp. impacting two soil materials of different textures were investigated. Aggregation was determined by measuring wet aggregate stability. Fungal growth was monitored using ergosterol and direct microscopic examination of hyphal length. Specifically, the objectives of this study were:

1. To determine the effect of alfalfa (*Medicago sativa* L.) residue, independent of biological activity, on soil aggregation
2. To determine if the addition of fungal inoculum into autoclaved soil increases soil aggregation.

MATERIALS AND METHODS

To accomplish the objectives of this laboratory experiment, a factorial design with two levels of fungi (inoculated and uninoculated), two levels of residue (alfalfa residue added and no crop residue added), two soils (Clarion loam and a silt loam parent material), and three replicates of each were used for a total of 24 jars.

Soil materials used in this experiment were basal loess parent material collected below an Ida silt loam (1.8-m depth; fine-silty, mixed, mesic, calcareous Typic Eudorthents) in western Iowa and a Clarion loam (0 to 15-cm depth; fine-loamy, mixed, mesic Typic Hapludolls) from the research farm near Ankeny, IA. These two soils were chosen based on their total C content and texture. The silt loam parent material consists of 78% silt. Because of the erosiveness of silt-size particles and the fact that western Iowa has some of the highest rates of soil loss in the world, the low C parent material was selected. The A horizon of soil mapped Clarion series was selected because it is very common in central Iowa and has a relatively high total C content (20.4 g kg^{-1}) in contrast with the silt loam parent material (1.4 g kg^{-1}). The soil material was air-dried and ground to pass a 2-mm sieve. A pressure plate apparatus was used to determine 33 kPa water contents.

Soil (400 g) was placed into 0.95-L (1 quart) Mason jars and capped with lids that contained a rubber stopper through which a Pasteur pipette was inserted. Glass wool was placed into the Pasteur pipette so that air could pass into the jar without contaminating the sample. All jars were autoclaved on 2 successive days for 30 min at 121 °C at 101.4 kPa pressure. Following the final autoclaving, all samples was aseptically brought to 33 kPa water content with sterile water. Sterilization of the samples was verified by sampling for CO_2, in the headspace of jars used as controls. Effect of alfalfa residue on soil aggregation was evaluated by adding 2 g of alfalfa that had been ground in a Wiley mill to pass a 2-mm screen. In treatments with alfalfa residue added, the residue was added to the soil and thoroughly mixed prior to autoclaving.

Samples were incubated at room temperature with normal fluorescent lighting for 7 d. Preliminary laboratory studies indicated that most of the aggregation occurs in the first 7 to 10 d of incubation. These results are similar to other published research studies evaluating biological contributions to soil aggregation (Molope et al., 1985; Molope, 1987).

Inoculum

A preliminary experiment using nine ergosterol-producing fungi indicated that *Chaetomium* sp. showed average response in terms of promotion of soil aggregation. *Chaetomium* sp. was grown in acidified potato dextrose broth for 7 d on a reciprocal shaker. Streptomycin sulfate (0.001 kg/L) and chloramphenicol (0.001 kg/L) were added to the cultures as bactericides. Prior to inoculating the jars, the cultures were examined for bacteria by direct microscopic counting. Cultures were placed into a sterile Waring blender and

blended at high speed for 1 min. Immediately after the blender was stopped, 1 mL of the chopped fungus was added to the autoclaved jars that were to be inoculated. Ergosterol content and hyphal length was measured on the inoculum.

In this study, wet aggregate stability was used, because it is a measure of soil erodibility by water (Yoder, 1936). Following the incubation period, the jars were sampled as follows. The soil in the jar was removed, quartered, and sampled for water content. A nest of sieves (8, 4, 2, 1, 0.5, 0.25, and 0.125 mm) were used in determining aggregate stability (Yoder, 1936) on 200 g of moist soil. The samples were immediately immersed and sieved, and no prewetting occurred. Wet sieving was completed on a sieving machine similar to the machine illustrated in Low (1954). The soil samples were immersed in water and sieved for 3 min at 130 cycles per min. Results were calculated using aggregate mean weight diameter (MWD) (Kemper & Rosenau, 1986).

Ergosterol and Hyphal Length

Ergosterol analyses followed the method developed by Grant and West (1986). Development of an ergosterol assay by Seitz et al. (1977) and its modification by Grant and West (1986) provided a relatively fast assay for determining live fungal biomass by quantifying soil ergosterol content (West et al., 1987). Ergosterol is the predominant sterol of most fungi (Weete, 1973; Grant & West, 1986; Davis & LaMar, 1992). Ergosterol correlates with fungal surface area (West et al., 1987), hyphal length (Matcham et al., 1985), and is more sensitive than the chitin or extracellular laccase assays (Matcham et al., 1985; Seitz et al., 1979). Therefore, ergosterol may be an important indicator of changes in the fungal portion of the soil microbial biomass.

Hyphal length was determined by direct microscopic observation of diluted soil samples using the membrane filtration technique (Hansen et al., 1974). Soil (1 g) was added to 500 mL of distilled and filtered water, then blended (Osterizer Pulsematic 14, Milwaukee, WI) at high speed for 1 min. A 1-mL aliquot was removed while operating the blender at low speed and placed into a filtering apparatus. Black polycarbonate filters (25 mm diam., 0.4 μm) (Poretics Corp, Livermore, CA) were used with 25 mm polyester drain disks placed under the polycarbonate filters. Vacuum was applied to filter the sample. Calcofluor white M2R (Sigma) fluorescent stain (1 mL of 0.002 g mL^{-1} distilled and filtered water) was expelled through a 0.2-μm syringe filter into the filtering apparatus and allowed to stand for at least 1 min (West, 1988). Vacuum was applied to pull the stain through the filter. The filter was washed with five, 1-mL aliquots of water. The filters were removed, placed on glass slides, and placed onto a slightly warm hotplate for approximately 10 min to dry the filter prior to mounting the cover slips. Cover slips were mounted over the filters using immersion oil. Hyphal length was determined at 400X using epifluorescence microscopy and a Nikon UV-1A filter cube on a Nikon microscope (Nikon Inc., Melville, NY). Hyphal length was calculated using the grid line intersect method developed by Olson (1950).

Homogeneity of variance was analyzed with Box-Cox transformations (Box et al., 1978, p. 231–238). Each significant ($P < 0.05$) variable with heterogeneous variance was transformed with the appropriate Box-Cox transformation and analyzed with the appropriate analysis of variance. Least squares analysis of variance was used on treatments with missing samples. Treatment differences ($P < 0.05$) were examined with planned contrasts.

RESULTS AND DISCUSSION

Autoclaved soil was used to determine effects of residue alone on soil aggregation. In autoclaved soil, the addition of ground alfalfa residue did not significantly increase soil aggregation (Table 16–1). Table 16–1 indicates that wet MWD decreased significantly in the Clarion loam soil. These results indicate that residue, in the absence of an active microbial component, does not increase aggregation. This decrease in aggregation may be due to the residue physically interfering with the effective attachment of individual soil particles.

Ergosterol and hyphal length measurements confirm that fungal growth occurred following inoculation of the samples (Table 16–2). The silt loam parent material had increased ergosterol content and a doubling of hyphal length in the inoculated samples when compared with the uninoculated samples. These increases, however, are much smaller than the increases found in the Clarion soil material. Addition of the fungal inoculum resulted in a ninefold increase in hyphal length. Large increases in hyphal length following addition of the inoculum to the Clarion soil not amended with alfalfa were observed. Autoclaving the Clarion soil may have liberated soil organic C, which could serve as substrate for the fungal inoculum.

Alfalfa residue additions to the silt loam parent material resulted in an 11-fold increase in hyphal length and a similar increase in ergosterol content (Table 16–2). Alfalfa additions to the Clarion soil material did not significantly increase aggregate stability (Table 16–3).

Increases in either soil ergosterol content or hyphal length corresponded with increased wet MWD (Tables 16–2 and 16–3). Increases in wet MWD

Table 16–1. Effects of alfalfa residue on soil aggregation using autoclaved soil.†

Residue added	Wet WMD‡
Silt loam parent material	
None	0.02a*
Alfalfa	0.06a
Clarion loam	
None	3.32a
Alfalfa	2.78b

* Means with the same letter are not significantly different ($P > 0.05$). Comparisons are between treatments of the same texture.
† Data are means of three replicates.
‡ Aggregate mean weight diameter.

Table 16-2. Fungal biomass as affected by residue or fungal inoculum addition.†

Soil	No fungal inoculum added		Fungal inoculum added	
	Ergosterol	Hyphal length	Ergosterol	Hyphal length
	mg kg^{-1}	m g^{-1}	mg kg^{-1}	m g^{-1}
Silt loam parent material				
Control	0.00a*	6.99a	0.79a	16.30a
Alfalfa	0.00a	20.46a	11.41b	182.89b
Clarion loam				
Control	0.07a	18.39a	1.54a	157.29a
Alfalfa	0.10a	29.56a	3.06a	233.82a

* Means with the same letter are not significantly different ($P > 0.05$). Comparisons are between treatments of the same texture within inoculum treatment.
‡ Data are means of three replicates.

Table 16-3. Effect of adding *Chaetomium* sp. and alfalfa residue on soil aggregation.†

Soil	Inoculated	No residue added	Alfalfa residue added
		Wet MWD‡	Wet MWD
		mm	
Silt loam parent material	No	0.02a*	0.06a
Silt loam parent material	Yes	0.22b	1.88b
Clarion loam	No	3.32a	2.78a
Clarion loam	Yes	4.43b	4.04b

* Means with the same letter are not significantly different ($P > 0.05$). Comparisons are between soil materials of the texture and residue treatment.
† Data are means of three replicates.
‡ Aggregate mean weight diameter.

were significantly correlated with increased hyphal length and soil ergosterol content (N.S. Eash et al., 1992, unpublished data). Inoculation of the autoclaved silt loam parent material resulted in a 10-fold increase in MWD of the soil aggregates. Aggregate MWD of the well-aggregated Clarion soil material significantly increased with the addition of the fungal inoculum.

The samples inoculated with *Chaetomium* sp. had significantly higher aggregate mean weight diameters as measured by wet sieving when compared with the autoclaved, uninoculated samples (Table 16-3). Addition of fungal inoculum to soil and the resultant growth of the fungi, as evidenced by increased ergosterol content and hyphal length, indicates that fungi are important mechanisms in aggregation.

Results from this experiment indicate that enhanced aggregation in soil following residue additions, as reported in other studies (Wilson & Browning, 1945; Oades, 1984), is not due to residue per se; rather, it is probably caused by greater fungal activity. Residue cover on the soil surface absorbs the energy of falling raindrops, but, perhaps more importantly, provides substrate for the soil fungi. By providing substrate, increased growth of fungal hyphae can physically stabilize soil particles into larger aggregates.

If soil aggregation cause-and-effect relationships were implied from past research data, tillage, rotation, or residue cover were often the likely reasons cited. This research does not dispute past observations; instead, it demonstrates quantitatively that soil fungi play a major role in soil aggregation. As we strive for a more sustainable future for our agricultural systems, the interactions of the fungi and soil management procedures are an important consideration.

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